United States Department of Agriculture
Agricultural Marketing Service
Science & Technology
Monitoring Programs Office
8609 Sudley Rd., Ste. 206
Manassas, VA 20110

April 23, 2007

TO: See Distribution List

FROM: Martha Lamont, Director
Monitoring Programs Office

SUBJECT: Microbiological Data Program Plan, April through June 2007

This Program Plan serves as the current Statement of Work for the period April 1, 2007, through June 30, 2007, for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

A. Program Status: The 2007 funding for the program has been released. MDP sampling and testing activities resumed in April 2007.

B. Personnel: Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

C. Summary Status: The 2006 MDP Progress Update and Data Summary was released via the Website in January 2007. The 2004 and 2005 MDP Progress Update and Data Summaries are also available on the MDP website at http://www.ams.usda.gov/science/MPO/Download.htm

D. Financial/Cooperative Agreements: MDP Cooperative Agreements for FY 2007 were issued March 23, 2007.

E. MDP Program Meetings: A Technical Meeting was held March 27-28, 2007 in Manassas, Virginia. Attendees included MDP Technical Program Managers (TPMs) and Quality Assurance Officers (QAOs) from all MDP participating States and staff from the Monitoring Programs Office. Program planning, technical, and quality assurance (QA) issues were addressed and are reflected in the Program Plan.

F. Equipment Purchases: MPO is purchasing the TEMPO® system for laboratories that did not purchase the instrument in 2006 – the Michigan, Minnesota, New York, Washington, and Wisconsin laboratories and the AMS National Science Laboratory (NSL).

G. Electronic Transfer of Data:

RDE Version Upgrades: A version upgrade to the Web-based RDE system was installed in April 2007 to make the data entry function for mPCR results available at all times and to increase the maximum size of the numeric value used to enumerate generic *E. coli*. With the next version upgrade, tentatively scheduled for December 2007, MPO plans to make password management more secure, increase the size of some text fields, and fix problems reported by laboratory users. A version upgrade to the RDE e-SIF system for laptops/palmtops was distributed in January 2007. With the next version upgrade, tentatively scheduled for June 2007, MPO plans to add a feature that will allow users to dynamically activate/add commodities on the commodity look-up table. MPO maintains a change request database to capture all problems identified and suggestions made regarding the RDE system.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling: Shipping Charts are distributed quarterly to Sampling Managers by MPO. The shipping chart covering the period April 1 through June 30, 2007 is attached to this document. Leaf and romaine lettuce are replaced with pre-cut (bagged) lettuce. Cantaloupe, tomatoes, green onions, and alfalfa sprouts continue. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). The samples collected in California will be sent to the following MDP laboratories: cantaloupes and tomatoes to NSL (US4), and bagged lettuce, green onions, and alfalfa sprouts to Ohio (OH4). Green onions and cantaloupes from Florida will be shipped to Colorado. Samples from all other States will be sent to the laboratory for that collection State.

B. Testing: Leaf and romaine lettuce are replaced with pre-cut (bagged) lettuce. Cantaloupes, tomatoes, green onions, and alfalfa sprouts will continue. The Ohio laboratory (OH4) will analyze all samples collected by Ohio and Maryland as well as bagged lettuce, green onions, and alfalfa sprouts collected by California. NSL (US4) will analyze all samples collected by Texas and cantaloupe and tomato samples collected by California. Colorado will analyze all samples collected by Colorado and cantaloupes and green onions from Florida. All other States will analyze samples collected in that State.

Testing Changes: Some method changes will be introduced based on special studies/validations performed during 2006, referenced literature, and discussions during the March Technical Meeting. These changes include:

(1) The addition of 0.1% Tween 80 to the UPB wash buffer for increased efficiency in removing bacteria from produce. This change is reflected in SOP MDP-LABOP-02 (Sample Receipt, Elution, Preenrichment, and DNA Extraction, Revision 8, 05/01/07).

(2) The replacement of the mechanical shaking procedure with a vigorous manual shaking step for cantaloupe, green onions, lettuce, and tomatoes. Alfalfa sprouts continue to be stomached. This change is reflected in SOP MDP-LABOP-02 (Sample Receipt, Elution, Preenrichment, and DNA Extraction, Revision 8, 05/01/07).

(3) Use of the soak method, developed by the U.S. Food and Drug Administration (FDA), for cantaloupes, lettuce, and tomatoes. These commodities will be soaked in UPB plus 0.1% Tween overnight. Alfalfa sprouts will continue to be stomached and green onions will continue to be surface-washed. For green onions and sprouts, any debris/plant material will be removed from the wash before overnight incubation. These changes are reflected in SOP MDP-LABOP-02 (Sample Receipt, Elution, Preenrichment, and DNA Extraction, Revision 8, 05/01/07).

(4) Introduction of the TEMPO® (*E. coli*) system for *E. coli* enumeration. This method will replace the current SOP MDP-MTH-01 ColiComplete method. All laboratories will perform a method verification study before using TEMPO EC®. This verification will consist of duplicate samples of bagged lettuce spiked at three levels. Colorado, Florida, and Ohio already have the TEMPO® instrument and will begin testing using the TEMPO® in April 2007. These laboratories will also perform a one-month side-by-side comparison with the ColiComplete method for all commodities collected by that State. Other laboratories will bring the TEMPO® online as instrument installation and method verification constraints allow. A 1:4 dilution of the wash sample will be used. This method is specified in SOP MDP-MTH-01A [Enumeration of *Escherichia coli* in Produce Samples by TEMPO® EC (*E. coli*) System, Original Version, 05/01/07]. A method verification for TEMPO system will be completed by May 25, 2007 and all laboratories will begin using TEMPO on June 1, 2007.

(5) All laboratories will capture total coliform bacteria for bagged lettuce. A new Organism/Test code of "TCO" has been activated in RDE so that a Total Coliform result can be reported for bagged lettuce samples only. Information detailing this change has been sent to all laboratories. Laboratories that do not yet have the TEMPO® system installed will use the ColiComplete method for *E. coli* and coliform bacteria determinations. This method is specified in SOP MDP-MTH-01B [Enumeration of Coliform Bacteria in Produce Samples by TEMPO® CC (Coliform Count) System, Original Version, 05/01/07].

(6) All laboratories will perform mPCR on all samples for detecting shiga toxin and enterotoxin-carrying pathogenic *E. coli*. NSL will purchase and mix 10 different primers at appropriate concentrations (10X) and distribute to all laboratories. FlashGel®, E-gel®, and other pre-cast gels may be used as an alternative to running regular agarose gel electrophoresis but laboratories must have a deviation on file.

(7) For isolation of *E. coli* O157:H7, mEC broth supplemented with novobiocin will be used with an additional enrichment at 42°C. All positive cultures will be subjected to immunomagnetic separation (IMS) and will be plated on different selective agar plates. This change is reflected in SOP MDP-MTH-06 (*Escherichia coli* O157:H7 in Fresh Produce by BAX® System, Revision 3, 05/01/07).

(8) For isolation of enterotoxigenic *E. coli*, the laboratories will include additional enrichment at 44°C using tryptone phosphate broth. This change is reflected in SOP MDP-MTH-07 [Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification, Revision 3, 05/01/07].

Target Microorganisms:

(1) *Escherichia coli* (*E. coli*): MDP laboratories will begin testing all samples for *E. coli* using the TEMPO® system. The TEMPO® offers a rapid, automated means to detect and enumerate generic *E. coli* without errors due to manual setup and bias in reading the fluorescence from positive culture tubes. Method procedures are detailed in SOP MDP-MTH-01A [Enumeration of *Escherichia coli* in produce samples by TEMPO® EC (*E. coli*) Method, Original Version, 05/01/07]. The TEMPO® will be validated by all MDP laboratories using spiked samples alongside program samples for one month in order to capture validation data for each commodity. A validation protocol will be supplied by MPO. A method verification for TEMPO system will be completed by May 25, 2007 and all laboratories will begin using TEMPO on June 1, 2007.

(2) Coliform Count: All laboratories will capture total coliform bacteria for bagged lettuce. Laboratories that do not yet have the TEMPO® system installed will use the ColiComplete method for *E. coli* and coliform bacteria determinations. This method is specified in SOP MDP-MTH-01B [Enumeration of Coliform Bacteria in Produce Samples by TEMPO® CC (Coliform Count) System, Original Version, 05/01/07].

(3) Pathogenic *E. coli*: MDP laboratories will screen all samples for pathogenic *E. coli* according to SOP MDP-MTH-07 [Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification, Revision 3, 05/01/07]. The mPCR assay tests for two types of pathogenic *E. coli*: (a) shiga toxin-producing *E. coli* (STEC) that carry genes coding for shiga toxins (Stx) 1 and 2 and (b) enterotoxigenic *E. coli* (ETEC) that carry genes coding for enterotoxins, heat labile (LT), and heat stable (ST) toxins.

(4) *Salmonella:* MDP laboratories will continue to screen all samples for *Salmonella* (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-04 (Detection of *Salmonella* in Fresh Produce by BAX® PCR, Revision 2, 01/01/06). Presumptive positive samples are subjected to enrichment and isolation as described in SOP MDP- MTH-03A (Isolation and Identification of *Salmonella* from Fresh Produce using Cultural Methods, Revision 1, 01/01/06).

(5) *E. coli* O157:H7: MDP laboratories will continue to screen all samples for *E. coli* O157:H7 (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-05 (Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® PCR, Revision 2, 01/01/06). Presumptive positive samples are subjected to IMS procedures and cultural confirmation, as described in SOP MDP-MTH-06 (*Escherichia coli* O157:H7 in Fresh Produce by BAX® System, Revision 3, 05/01/07).

C. Quality Assurance:

Proficiency Testing Program: The next proficiency testing (PT) round will be introduced in July/August 2007. The test organism will be generic *E. coli* with testing to be performed using the TEMPO ® EC method.

SOPs: The following new and revised SOPs were issued during April 2007, and reflect the changes noted under Section (B), Testing, of this document:

- SOP MDP-LABOP-02: Sample Receipt, Elution, Preenrichment, and DNA Extraction (Revision 8, 05/01/07)
- SOP MDP-MTH-01A: Enumeration of *Escherichia coli* in Produce Samples by TEMPO® EC (*E. coli*) System, Original Version, 05/01/07)
- SOP MDP-MTH-01B: Enumeration of Coliform bacteria in Prodcue Samples by TEMPO®CC (coliform Count) System, Original Version, 05/01/07 – for enumerating coliform bacteria in pre-cut bagged lettuce.
- SOP MDP-MTH-06: MDP-MTH-06 (*Escherichia coli* O157:H7 in Fresh Produce by BAX® System, Revision 3, 05/01/07).
- SOP MDP-MTH-07: MDP-MTH-07 [Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification, Revision 3, 05/01/07].

SOPs are posted to the MDP website when distributed to program participants. Refer to: http://www.ams.usda.gov/science/MPO/SOPs.htm.

D. Archiving and Additional Testing:

Archival of Isolates: NSL (US4) serves as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories: All target organisms are frozen in Microbank™ vials and shipped to NSL (US4). Vials are shipped by NSL (US4) to the FDA/Center for Veterinary Medicine (CVM) laboratory in Laurel, Maryland for antimicrobial resistance testing for inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed-field gel electrophoresis (PFGE) analysis for inclusion in PulseNet. *Salmonella* and *E. coli* O157 isolates are also serotyped by FDA/CVM. Pathogenic *E. coli* isolates are shipped by NSL (US4) to Pennsylvania State University for serotyping.

E. Transfer of Data: AMS transfers data to the Centers for Disease Control and Prevention (CDC) and FDA on a semi-annual basis.

F. Future Program Directions:

Shigella: Two MDP laboratories evaluated the *Shigella* method developed by the Division of Consolidated Laboratory Services (DCLS). The laboratories noted problems with the 16S rDNA internal control. *Shigella* will not be introduced in 2007; however, if 2008 funds are restored this issue may be revisited. MDP has been participating in the FERN/FSIS and LRN/CDC discussions on selecting a robust *Shigella* realtime PCR method for Smart Cycler platform. FERN has begun a validation study and has selected some of the MDP laboratories and NSL for participation. MDP will follow the progress on this validation process.